Decreasing Analysis Time Using Poroshell 300SB-C18 in Analysis of a Protein Digest

Application

Proteomics

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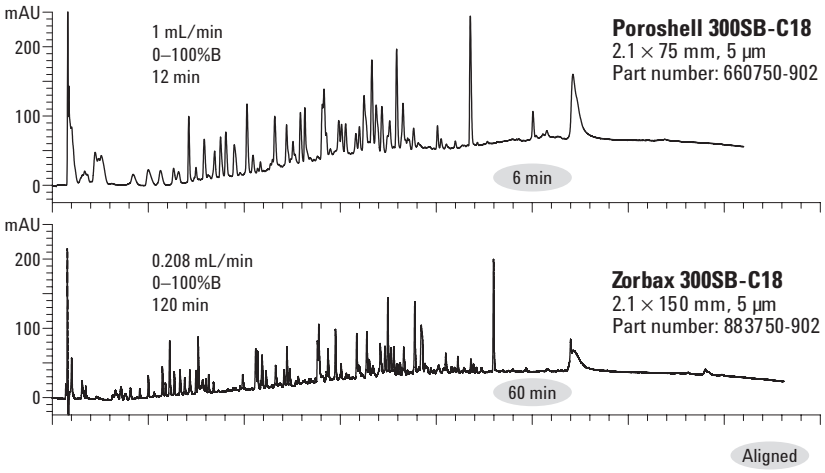
In the modern protein chemistry laboratory or core facility, time is literally money. Time wasted cannot be recovered, so it is best to avoid that situation. Poroshell 300SB-C18 enables the protein analyst to shorten peptide mapping time by a factor of nine, as may be seen in the chromatograms below.



Conditions

Instrument	Agilent 1100 binary system
Column	300SB-C18 (as indicated above)
Mobile phase	A = 95% H_2O, 5% ACN, 0.1% TFA B = 5% H_2O, 95% ACN, 0.07% TFA
Flow	As above
Piston stroke	20 µL
Detection	UV: 215 nm
Temperature	70 °C
Agilent 1100 well plate autosampler with delay volume reduction	
Injection volume	20 µL (0.22 µg/1 µL)
Sample	BSA Tryptic digest (15 hours, 70 pmol)

While a large number of peaks are resolved in both chromatograms above, it is apparent that the Poroshell column shows reduced retention for smaller peptides (those toward the far left). This does not pose a problem when using this technique for protein identification, where very short peptides are rarely informative.

The Poroshell column also performed well when using acetic acid to replace TFA for liquid chromatography/mass spectrometry (LC/MS) analysis. As might be expected, there was slight broadening of peaks, but runtimes were still very short (<6 min).

Highlights

- Poroshell was designed with the needs of the protein chemist in mind — high speed, high sensitivity, and superior resolution in one small package.
- The sterically protected bonded phase used in Poroshell SB products provides the exceptional chromatographic stability desirable for typical low-pH peptide/protein analysis.
- Because of the thin superficially porous layer, Poroshell 300SB-C18 allows rapid equilibration with large molecules having slower diffusion into pores.
- Poroshell 300SB-C18 allows the use of higher linear velocities, with no loss in resolution. This translates into very short run times and high-throughput analysis.
- Poroshell columns were designed with a 2.1-mm id so that high linear velocities could be achieved at typical flow rates; 1 mL/min on this column compares to nearly 5 mL/min on a conventional 4.6-mm id column.

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